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TRANSACTIONS

Oryzanin "Antineuritic Vitamin". IV.

On the Activity and Thermostability of Oryzanin Hydrochloride.

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The Conference[1] on Vitamin Standards, held in London in 1931 under the auspieces of the League of Nations Health Organisation, has adopted, as international standard, the adsorption product of Vitamin B_1 prepared at the Medical Laboratory, Batavia, by the method of Seidel, as described by Jansen and Donath, and recommended 10 mg. of this product as the International Standard Unit of Vitamin B_1 activity. This standard product is prepared by extracting rice polishings continuously for two days with water, sufficient sulphuric acid being added to make the pH 4.5. Salicylic acid to a concentration of 0.2 per cent and toluene are added to prevent bacterial decomposition. For each 100 kg. of the original rice polishings, 3 kg. of Fuller's earth are added to the extract which is then stirred for 24 hours. Subsequently, the solution is filtered off and the Fuller's earth, after being washed with water and alcohol, is dried; 3 kg. of the Fuller's earth adsorbate represents the Vitamin B_1 from 100 kg. of rice polishings.

Quite recently, the isolation of crystalline vitamin B_1 has been carried out by various authors, but the chemical composition as well as the physiological activity of these preparations are not yet quite conformable, as shown in the following table;

Authors	Material (Yield)	Chemical composition	Activity
Jansen & Donath[2]	Rice polishings (100 mg from 300 kg)	$C_6H_{10}N_2O$	Bondol ·····3~4 γ. Pigeon ·····12~60 γ.
Jansen, Kinnersley, Peters & Reader[3]	Rice polishings & yeast	——	Pigeon's day dose ···7~9 γ. Rat's day dose ·····5 γ.

Jansen, Wibaut, Huber & Wiardi[4]	Rice polishings & yeast	$C_{12}H_{18}N_4SO_2$	——
Windaus et al.[5]	Yeast (70~80 mg. from 100 kg)	$C_{12}H_{16}N_4SO+H_2O$	Pigeon's day dose ·····2.4 γ.
Ohdake[6]	Rice polishings (1.6 g from 11.500 kg Yeast (activated) (0.15~0.25 g from 20 kg)	$C_{12}H_{16or18}N_4SO_2$	Pigeon's curative dose ···5 γ. Pigeon' day dose ·····2.5 γ. Rat (international standard unit) ·····1 γ.
Van Veen[7]	Rice polishings	$C_{12}H_{20}N_4SO_2$	Rice birds·····1.6~2 γ. Pegeon's day dose···8~12 γ. Rat's day dose·····3~6 γ. Rat (international standard unit) ·····4~8 γ.
Kinnersley et al[8]	Yeast (500 mg from 2.000 kg)	——	Pigeon's day dose (per os) ·····1.6~2.8 γ.
Seidel[9]	Yeast	Picrolonate	Rat's curative dose·····15 γ.
Williams, Waterman & Keresztesy[10]	Rice polishings (5 g from 1 ton.)	——	Rat's day dose ·····0.8 γ.?

It can be seen from the above table that there are still remarkable differences in the activity of the different preparations. This might be partly due to the inactivation of B_1 during the isolation process and partly to the technique of biological tests.

From such a reason, the Second Conference[11] held in London in 1934 has proposed to compare the potency of these crystalline preparations with the standard product with the aim of ultimately adopting pure crystalline B_1 as the international standard.

The present author has already reported in the previous papers[6] that 0.001 mg of his oryzanin hydrochloride, isolated from rice polishings and from yeast is the minimum for maintaining normal growth of a young albino-rat and is equivalent to the international standard unit. The "day dose"[12] for a pigeon (ca. 300 g) is 0.0025 mg. and the pigeon's curative dose 0.005 mg. But the material being not sufficient for thorough investigation at that time, so the author has repeated the isolation of the crystals from rice polishings as well as from yeast with the purpose of obtaining more material. The yield has now been much improved and at present, the author is able to obtain 0.5~1.0 g. of pure crystals from 1,000 kg. of rice polishings, 2.4 g. from 100 kg. of pressed activated yeast (=ca. 23 kg. dry yeast) respectively. The chemical composition as well as the biological activity of these refined preparations have been proved to be the same as reported in the previous paper, except a little decrease in the activity of the oryzanin hydrochloride, regenerated from the picrolonate.

The activity was tested on young albino rats fed on an artificial diet

deficient in vitamin B_1 and the result was compared with the international standard product as follows:

(1) When young albino rats of about 45 g. were fed on the artificial diet,[13] deficient in vitamin B_1, consisting of 60% purified starch, 20% purified casein, 15% peanuts oil and 5% McCollum' salt mixture (No. 185), supplemented daily with three drops of cod-liver oil and 0.4 g. of autoclaved yeast, they developed the symptoms of B_1 deficiency usually in 4~5 weeks. (Chart. 1)

(2) Young rats fed on the same artificial diet, when supplemented daily with 10 mg. of the standard adsorption product[14] from beginning of the experiment, maintained normal growth, gaining about 10 g. per week in average for 5~6 weeks. When supplemented with 20 mg. daily, the growth was still better. (Chart. 2)

(3) Rats fed on the same artificial diet, supplemented daily with 0.0005 mg. of oryzanin hydrochloride isolated from rice polishings, developed the symptoms of B_1 deficiency in 4~5 weeks. By supplementing daily with 0.0008 mg, they were perfectly healthy during 6~7 week, though it was still insufficient to maintain the normal growth. (Chart. 3)

(4) With a daily dose of 0.001 mg, young rats maintained normal growth, gaining about 10 g. per week in average during 6 weeks. When supplemented daily with 0.0012~0.0015 mg, nearly the same results were obtained. Thus we see that 0.001 mg. is the minimum for the maintenance of the normal growth of a young rat and 0.001~0.0015 mg. are equivalent to the standard unit. (Chart. 4)

(5) By giving daily 0.002, 0.003, 0.005 and 0.01 mg. respectively, the rats showed better growth for 6~7 weeks, gaining 11~14 g. per week in average and the results can be compared with 20 mg. of the standard product. (Chart. 5)

(6) The hydrochloride, isolated from yeast gave also the same result, a daily dose of 0.001 mg. being required to maintain the normal growth. (Chart. 6)

(7) Young rats developed the severe symptoms of B_1 deficiency by feeding on the same artificial diet. When supplemented daily with 0.001 mg of the hydrochloride isolated from rice polishings as well as from yeast, they were cured in two days and regained the normal growth. With daily dose of 0.0015, 0.005, 0.01 mg respectively, the rats showed better growth rate after cure. So it can be assumed that 0.001 mg. is the minimum dose for curing the young rat of B_1 deficiency and for maintaining the normal growth. (Chart. 7)

(8) In the previous paper[6] the author has reported that the hydrochloride, regenerated from the picrolonate, showed the same elementary

composition and possessed the same properties with the original hydrochloride. But it has been revealed in the present experiment that a daily dose of 0.0015 mg. of the same preparation is required at least for the normal growth, so it must be assumed that about 30% of the activity has been lost during the regeneration process from the picrolonate. (Chart. 8 & 9)

(9) "Injectio Oryzanin Fortior" of Sankyo & Co. is the vitamin B_1 preparation containing in 1 cc. 0.5 mg of B_1 hydrochloride isolated from rice polishings. With a daily dose of 0.002 cc. of this solution (=0.001 mg of the hydrochloride) young rats showed normal growth and 0.003 cc. (=0.0015 mg) gave nearly the same result while 0.0008 mg were proved to be insufficient. So it can be assumed that 0.001~0.0015 mg are equivalent to the standard unit. (Chart. 10)

TABLE II.—*Albino-rats fed on the artificial diet with oryzanin hydrochloride,*

Material	Dose (mg)	Number of Rats	Growth rate (per week) Average (5 weeks) (g)	1st. week (g)	5th. week (g)
Standard adsorption product	10.0000	6	10.9	12.3	8.6
	20.0000	1	13.0	18.0	10.0
Oryzanin hydrochloride from rice polishings	0.0005	4	——	B_1-deficiency	——
	0.0008	4	4.0	10.0	1.6
	0.0010	6	10.4	11.4	8.4
	0.0012	4	8.4	8.4	12.2
	0.0015	3	9.6	15.1	6.4
	0.0020	2	11.2	16.5	7.8
	0.0030	2	13.8	15.0	12.0
	0.0050	2	13.8	9.5	16.7
	0.0100	3	13.8	14.8	17.3
Oryzanin hydrochloride from yeast	0.0007	2	——	——	——
	0.0008	2	5.1	8.5	3.6
	0.0010	5	7.6	15.1	5.7
	0.0012	3	7.8	9.7	7.8
	0.0015	2	9.3	14.3	5.3
	0.0030	1	14.3	14.5	12.0
Oryzanin hydrochloride regenerated from the picrolonate	0.0008	3	——	B_1-deficiency	——
	0.0010	6	5.1	11.9	0.4
	0.0012	4	4.9	14.1	3.8
	0.0015	2	6.9	11.5	5.8
	0.0020	1	8.0	11.5	2.5
"Injectio Oryzanin Fortior" of Sankyo & Co.	0.0008	4	2.5	9.4	1.6
	0.0010	6	8.4	9.8	8.2
	0.0015	2	10.7	19.8	7.5

The above results show that 0.001 mg. of oryzanin hydrochloride isolated from rice polishings as well as from yeast is the minimum for maintaining the normal growth of a young albino rat and 0.001~0.0015 mg. are equivalent to the standard unit. The results of pigeon's test with oryzanin hydrochloride will be reported later on.

Thermostability of Oryzanin hydrochloride :-

It is known that the antineuritic vitamin is thermolabile. Chick and Hume[15] have observed that the activity of yeast extract is reduced to ca. 50% by heating for 1 hour at 100°C and to ca. 30% by heating for 2 hours at 122°C. The present author has studied on the thermostability of the oryzanin hydrochloride from rice polishings in crystalline state as well as in aqueous solution.

(A) Aqueous solution ; Each 0.01% aqueous solution, heated for one hour at various temperature, was tested on young albino rats fed on the same artificial diet as described in the previous part.

1) When heated for 1 hour at 110°C, there was no loss on activity, showing the normal growth with a daily dose of 0.001 mg. (Chart. 11)

2) Heated for 1 hour at 120°C, a daily dose of 0.002 mg. was required for the normal growth and consequently the loss on activity is estimated to be about 50%. (Chart. 11)

3) Heated for 1 hour at 130°C, a daily dose of 0.01 mg was required. The loss on activity is therefore approximately 90%. (Chart. 12)

4) Heated for 1 hour at 140°C, it was inactive even with a daily dose of 0.01 mg. (Chart. 12)

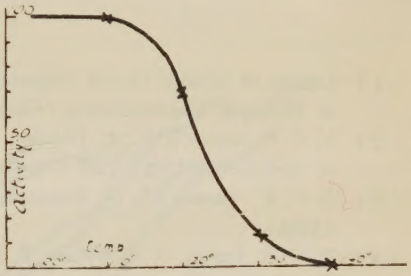


Fig. 1. *Thermostability of Oryzanin hydrochloride in aqueous solution.*

The above results are shown in Fig. 1.

(B) Crystals of oryzanin hydrochloride ;

1) Heated for 1 hour at 170°C, a daily dose of 0.005~0.01 mg was required for normal growth and the loss of activity was about 80%. (Chart. 13)

2) Heated for 1 hour at 180°C, it was nearly inactive even with 0.02 mg daily. (Chart. 14)

TABLE III.—*Albino rats fed on the artificial diet with oryzanin hydrochloride heated at various temperatures.*

Material	Temp. (1 hr) °C	Dose (mg)	Number of rats	Growth rate (per week) Average (5 weeks) (g)	1st week (g)	5th week (g)
Aqueous solution of Oryzanin hydrochloride	110	0.001	2	10.5	8.8	12.5
	120	0.001	1	6.5	11.0	12.0
		0.002	3	10.8	10.5	13.2
	130	0.001	2	——	B_1-deficiency	——
		0.002	1	——	——	——
		0.005	2	5.6	——	——
		0.010	1	16.2	——	——
	140	0.010	2	——	B_1-deficiency	——

Crystals of Oryzanin hydrochloride	170	0.001	2	——	B_1-deficiency	——
		0.002	3	8.1	8.7	3.7
		0.005	3	9.2	6.8	7.5
		0.010	2	15.2	13.5	14.0
	180	0.005	3	5.6	7.3	3.8
		0.010	1	6.5	6.5	5.5
		0.020	2	7.6	5.0	5.3

From the above results, it can be seen that the activity of oryzanin hydrochloride is practically lost by heating the aqueous solution for one hour at 140°C and the crystals for one hour at 180°C.

The authors wish to express their sincere thanks to Prof. Dr. U. Suzuki for his kind advice throughout the work and to Sankyo & Co. for the kind supply of the material.

(Tokio, Jan. 30. 1935)

Literature

(1) League of Nations Health Organisation (1931). Report of the Permanent Commision of Biological Standardisation (Geneva).

(2) B. C. P. Jansen & W. F. Donath: Mededeelingen van den Dienst der Volksgezondheid in Ned-Indie, (1927) Part I. 1~5.

(3) B. C. P. Jansen, H. W. Kinnersley, R. A. Peters & V. Reader: Bioch. J., **24**, 1824 (1930).

(4) B. C. P. Jansen, J. P. Wibaut, P. J. Hübers & P. W. Wiardi: Rec. Trav. chem. Pay-Bas, **52**, 366 (1933).

(5) A. Windaus, R. Tschesche, H. Ruhkoph, F. Laquer & F. Schultz: Nachrich. Göttingen, 207~213 (1931); A. Windaus, R. Tschesche & H. Ruhkoph; Nachrich. Göttingen, 342~346 (1932); Z. Physiol. Chem., **204**, 123 (1932).

(6) S. Ohdake: Proc. Impr. Acad. Japan, **7**, 102~105 (1931); Ibid., **8**, 179~182 (1932); Ibid., **10**, 95~98 (1934); Bull. Agric. Chem. Soc. Japan, **8**, 11~46 (1932); Ibid. **8**, 111~121 (1932); Ibid., **10**, 71~76 (1934).

(7) A G. VanVeen: Medeelingen van den Dienst der Volksgezondheid in Ned-Indie, (1932); Z. Physiol. Chem. **208**, 125~128 (1932); Rec. Trav. Chem. Pay-Bas, **52**, 366 (1933).

(8) H. W. Kinnersley, J. R. O'Brien & R. A. Peters: Biochem. J. **27**, 232~239 (1932).

(9) A. Seidel & M. I. Smith: J. American Chem. Soc., **55**, 3380 (1933).

(10) R. R. Williams, R. E. Waterman and J. C. Keresztesy: J. American Chem. Soc., **56**, 1187~1191 (1934).

(11) League of Nations Health Organisation (1934). Report of the Permanent Commission of Biological Standardisation (Geneva).

(12) Kinnersley & Peters: Bioch. J., **19**, 820 (1925).

(13) Chick & Roscoe: Bioch. J., **22**, 790 (1928).

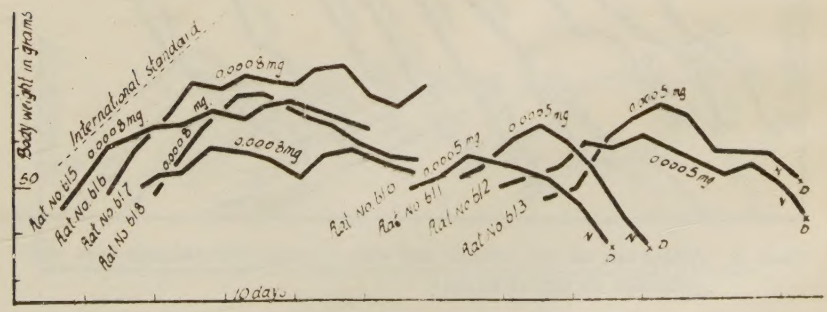
(14) Standard Adsorption Product of B_1, Prepared by the National Institute for Medical Reseach, London.

(15) Chick & Hume: Proc. Roy. Soc. London, **90**, 44 (1917).

N……B_1-deficiency D……Dead

Chart 1. Albino-rats on the artificial diet. (Control)

Chart 2. Albino-rats on the artificial diet with the international standard product.



N……B_1-deficiency D……Dead

Chart 3. Albino-rats on the artificial diet with oryzanin hydrochloride from rice-polishings (0.0005~0.0008 mg).

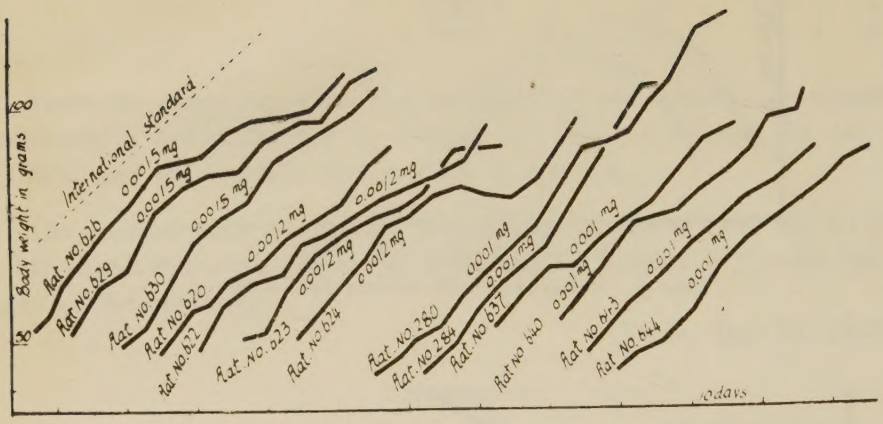


Chart 4. Albino-rats on the artificial diet with oryzanin hydrochloride from rice-polishings (0.001~0.0015 mg).

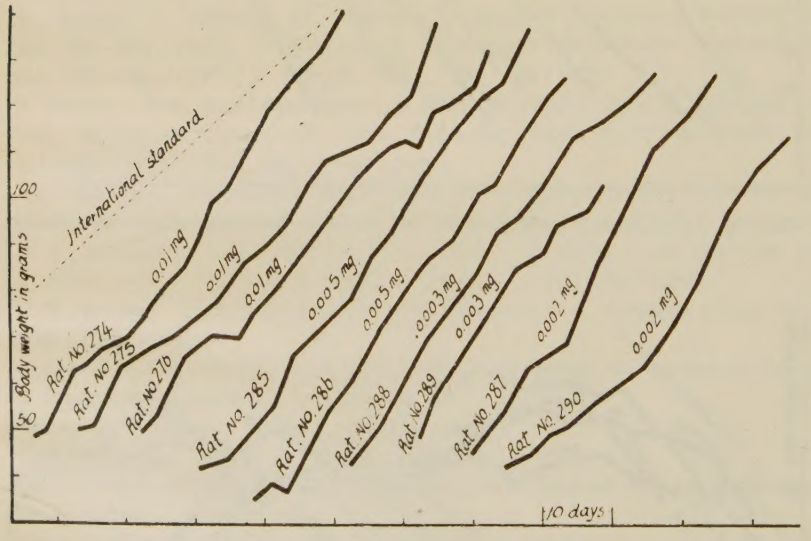


Chart 5. Albino-rats on the artificial diet with oryzanin hydrochloride from rice-polishings (0.002~0.01 mg).

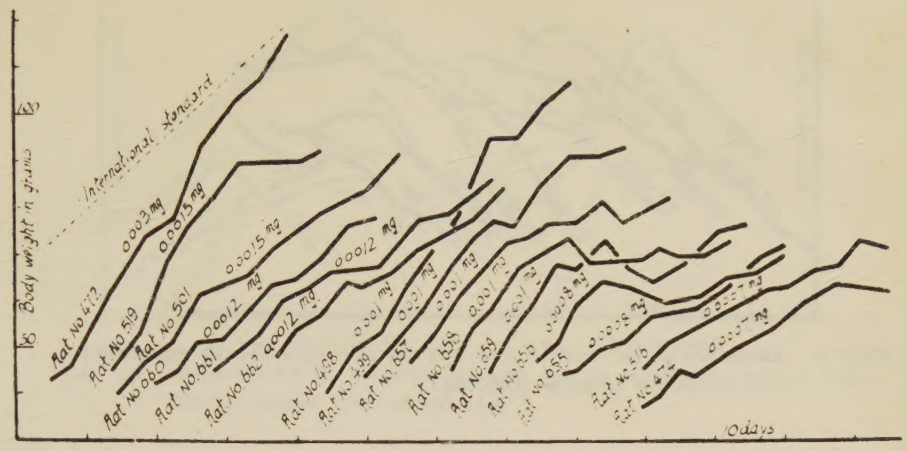
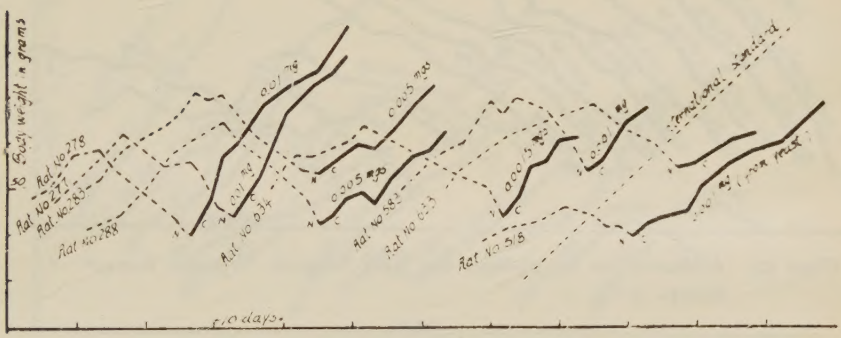
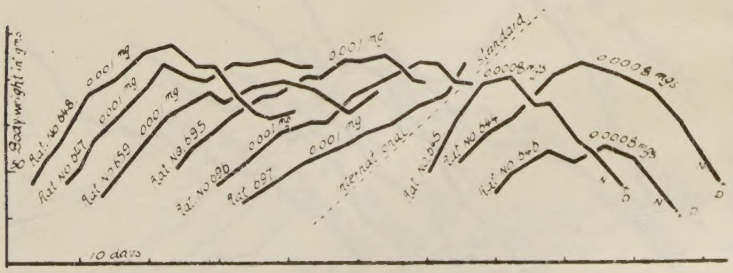


Chart 6. Albino-rats on the artificial diet with oryzanin hydrochloride from yeast.



N……B_1-*deficiency* *C*……*Cured*

Chart 7. Albino-rats on the artificial diet with oryzanin hydrochloride (curative test).



N……B_1-*deficiency* *D*……*Dead*

Chart 8. Albino-rats on the artificial diet with oryzanin hydrochloride regenerated from picrolonate (0.0008～0.0.1 mg).

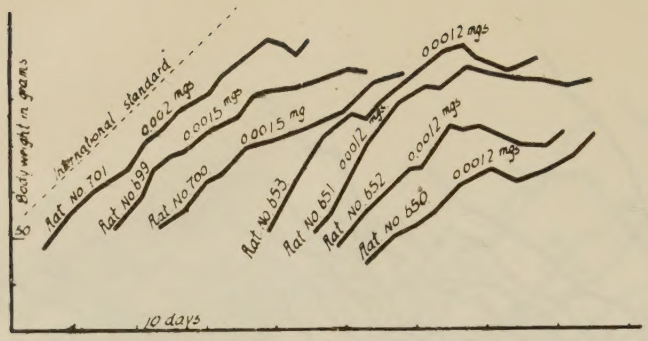


Chart 9. Albino-rats on the artificial diet with oryzanin hydrochloride regenerated from picrolonate (0.0012~0.002 mg).

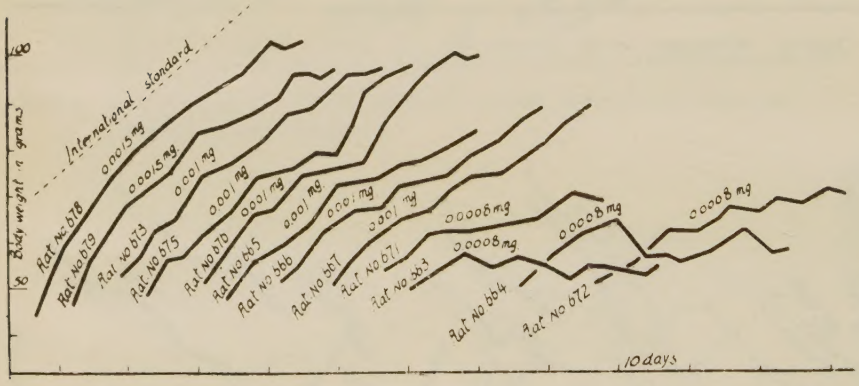


Chart 10. Albino-rats on the artificial diet with "Injectio Oryzanin Fortior" of Sankyo & Co.

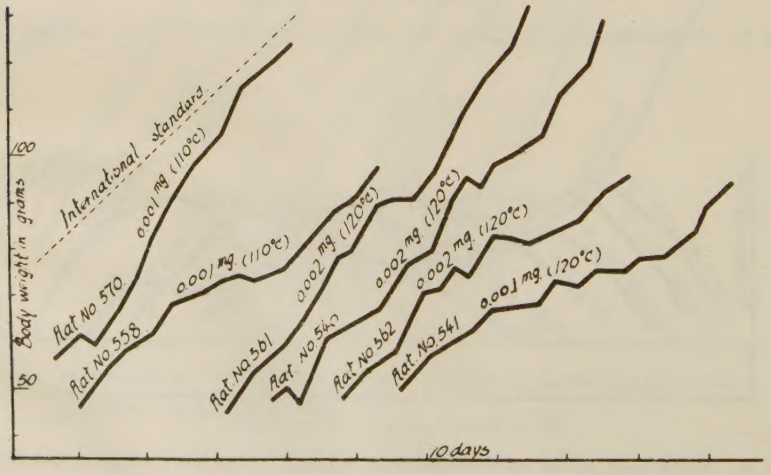
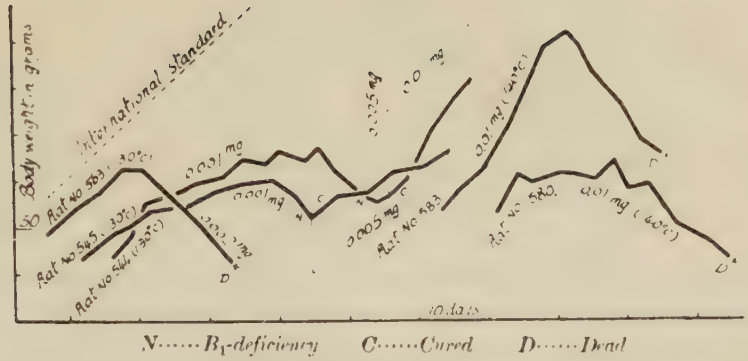
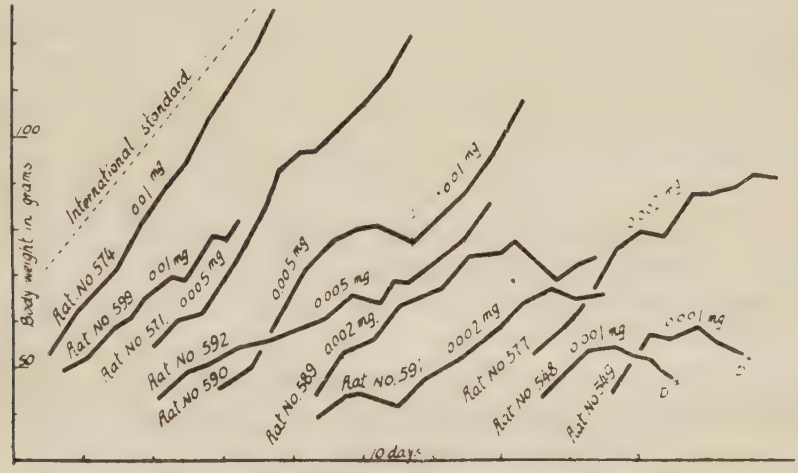


Chart 11. Albino-rats on the artificial diet with aqueous solution of oryzanin hydrochloride, heated 1 hr. at 110~120°C.



N……B_1-deficiency C……Cured D……Dead

Chart 12. Albino-rats on the artificial diet with aqueous solution of oryzanin hydrochloride, heated 1 hr. at 130~140°C.



N……B_1-deficiency D……Dead

Chart 13. Albino-rats on the artificial diet with crystals of oryzanin hydrochloride, heated 1 hr. at 170°C.

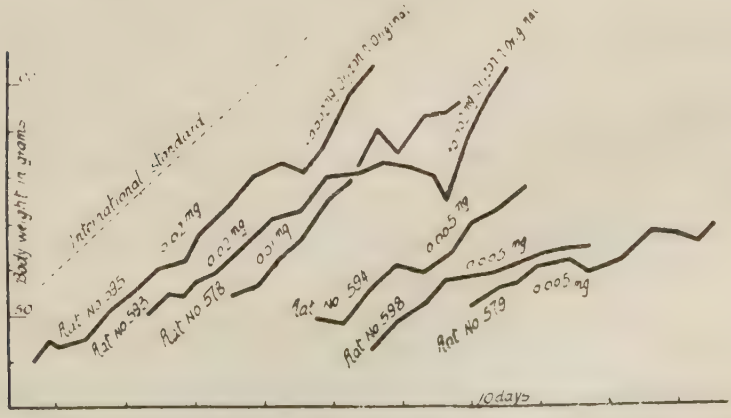


Chart 14. Albino-rats on the artificial diet with the crystals of oryzanin hydrochloride, heated 1 hr. at 180°C.

Studies on Amylosynthease.

Part II.—Amylosynthease of rice.

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(Received February 12, 1935.)

(1) 500 g of finely powdered polished rice were washed with cold water, macerated with 2 volumes of 5% K_2SO_4 solution for 2 days and washed again with water. The powders thus treated were removed into an 1 l. flask and were digested with 1 or 2 g of papain (1 : 1000) at 25°C for a day, the same volume of water and a little toluene having been added. The digests were filtered, the filtrate was examined for its enzymatic power and a strong action of amylosynthease has been detected.

(2) Flocculent precipitates, which carried down a greater part of amylosynthease, have been obtained by saturating the enzymatic solution, prepared as refered to above, with ammonium sulphate. By repeating the dissolving into water and the reprecipitating with ammonium sulphate, the enzyme preparation was purified to a certain degree.

(3) Amylosynthease preparation obtained by precipitating with ammonium sulphate, was precipitatable by adding alcohol or aceton to the extent of 80%, to aqueous solution, in contradistinction to yeast amylosynthease. The precipitates were colourless powder with silky luster and had remarkable activity.

(4) Thus, by using polished rice the author was able to prepare a strong amylosynthetic but weak amylolytic enzyme solution whereas when unpolished rice was used the reverse was the result. From rice polishings and embryos an amylase preparation was obtained which had shown no sign of the presence of amylosynthease.

(5) The amylosynthease of rice was precipitated by mercuric chloride or by lead acetate and it was recovered by treating the precipitates with H_2S gas.

(6) The enzyme is also adsorbed by aluminium hydroxide, Ca-phosphate, ferric oxide and caolin and eluted by Na-bicarbonate.

(7) The optimum temperature lies between 35～46°C, while the optimum pH was 6.2.

(8) As the above results show the amylosynthease of rice behaves quite differently from that of yeasts.[1], [2], [3], [4]

	Rice amylosynthease	Yeast amylosynthease
$(NH_4)_2SO_4$	Precipitated by the reagent reversibly	The precipitation cannot be repeated more than once
Alcohol or aceton	Precipitated	Not precipitated
H_2S gas	Not harmful	Harmful
Hg-chloride	Recovered	Not recovered
Cd-chloride	Not precipitated	Precipitated
Glycerine	Well soluble	Insoluble
Optimum temperature	25～27°C	35～46°C

(9) Amylosynthease similar to that of rice was also detected in corn, millets, potato and seed of panic-grass.

(10) The author examined the relative velocities of polymerisation of different dextrines by the action of rice amylosynthease. The results are shown in Chart 1.

The temperature effects upon the action of the same enzyme are drawn in Chart 2.

It will be observed from the charts, that the action of rice amylosynthease is stronger than that of yeasts.

(11) The Chart 3 shows the effect of the admixture of amylases of various origin to the amylosynthease solution and while Chart 4 shows that of the reverse case. In both cases, the two kinds of the enzymes behave antagonistically to each other. The antagonistic action increases proportionally with the amount of enzyme, finally the action of either one of the enzymes disappearing when a sufficient amount of the revial enzyme is used.

(12) A 5% solution of K_2SO_4 was used to remove amylase as well as its zymogen from polished rice. The same purpose is attainable by treating the material with Na_2SO_4, KCl or NaCl solution of the same concentration. A M/50 solution of KCN, Na-sulphite or Na-hyposulphite may, also, be used to the same effect.

During the treatment the amylosynthease remained intact in the material. When the polished rice powder washed with the salt solution, was digested with papain, the amylosynthease became first active and soluble.

It is likely, therefore, that the amylosynthease exists in rice as its zymogen, perhaps in the form of amylosynthease-protein-complex.

Literature

(1) T. Minagawa: Bull. Agr. Chem. Soc. Japan, **34**, 10 (1934).
(2) " : Proc. Imp. Acad. Japan, **97**, IX, No. 3 (1933).
(3) " : " **244**, VIII, No. 6 (1932).
(4) " : " **258**, VII, No. 7 (1931).

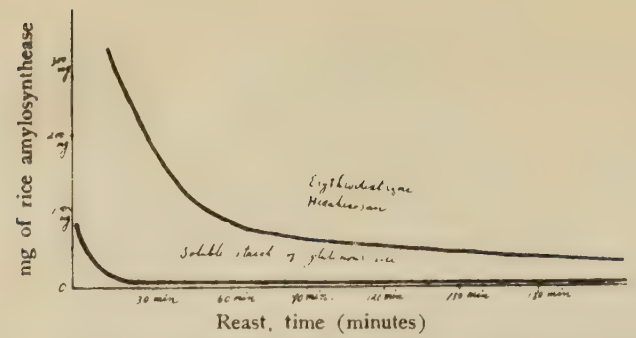

Chart 1. 40 cc of 1% solution (Erythrodextrine, Hexahexosan or soluble starch of glutinous rice) is added with rice amylosynthease and the times required untill the solution gives blue colour with iodine are estimated (at room temperature).

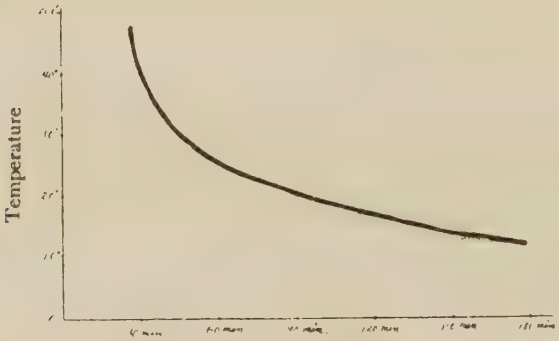

Chart 2. 40 cc of 1% solution of erythrodextrine is added with 50 mg rice amylosynthease and the times required until the solution gives blue colour with iodine are estimated.

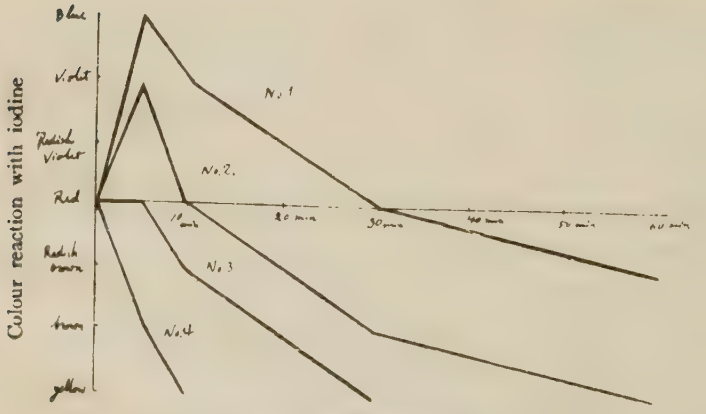

Chart 3 A. 20 cc of 1% solution of soluble starch of glutinous rice is added with 50 mg of rice amylosynthease and 0.4 mg Takadiastase (No. 1); 0.8 mg Takadiastase (No. 2); 1.6 mg Takadiastase (No. 3); 3.2 mg Takadiastase (No. 4) and the changes of colour reaction with iodine are described.

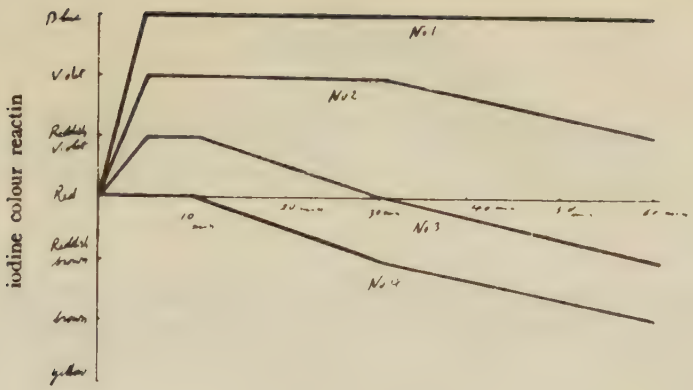

Chart 3 B. 20 cc of 4% solution of soluble starch of glutinous rice is added with the same preparations as chart 3 A.

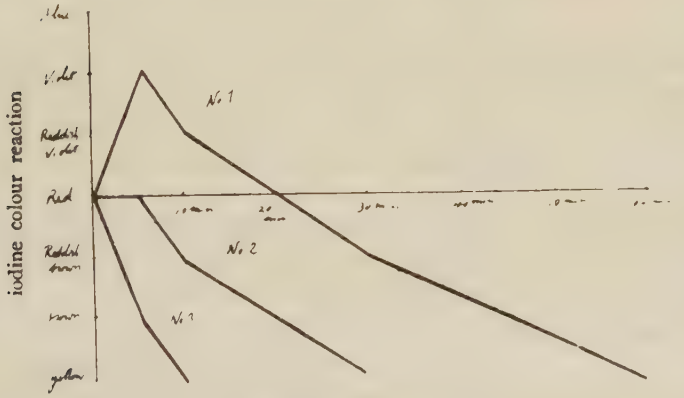

Chart 3 C. 20 cc of 1% solution of soluble starch of glutinous rice is added with 50 mg of rice amylosynthease and 5 mg Pancreatic amylase (No. 1); 10 mg Panc. amylase (No. 2); 20 mg Panc. amylase (No. 3) and the colour reactions are traced.

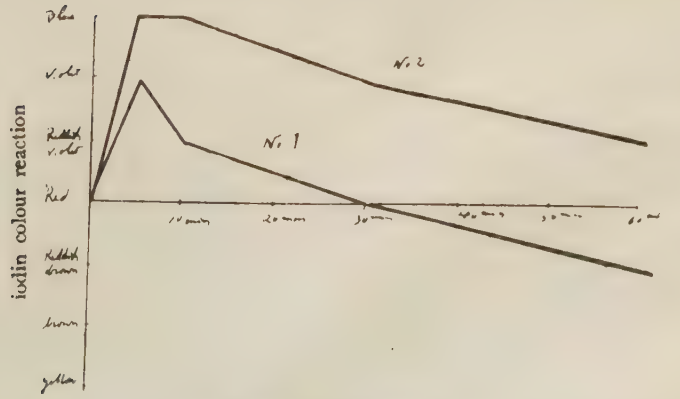

Chart 4. 20 cc of 1% solution of soluble starch of glutinous rice is added with 0.5 mg Takadiastase and 45 mg amylosynthease (No. 1); 90 mg amylosynthease (No. 2) and the changes of colour reaction are described.

ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

Influence of Deficiency of Three Essential Elements (N, P, K) on the Yield, Ash-constituents (Si, Ca, P,K) and Nitrogen Content of Unhulled Rice. (Continued) (pp. 255～260): By Chikafumi ICHIKAWA. (Agr. College of Gifu, Japan.)

Polysaccharide (IX).—Über die Hydrolyse des neues Kohlenhydrats "Xyloglukuronid" und über die Isolierung des neues Disaccharids "Xyloglukuronsäurebarium". (pp. 261～271): Von Kitsuzi NISHIDA und Hideo HASHIMA. (Holzchemisches Institut, Kyushu Kaiserliche Universität, Fukuoka, Japan.)

Ein eingehender Weg zur Klärung der Frage, ob das neue Kohlenhydrat „Xyloglukuronid"[1] aus 1-Mol Xylose und 1-Mol Glukuronsäure aufgebaut ist und ob sich darin Glukose, Mannose und noch andere Hexosen oder Pentosen vorfinden, scheint uns die theoretisch quantitativ durchgeführte Hydrolyse zu sein. Zu dieser Prüfung des polymeren Kohlenhydrats sind von verschiedenen Forschern verschiedene Reagentien festgelegt worden, und als geeignete Mittel dienen hochkonzentrierte Salzsäure und verdünnte Salz-, Salpeter- und Schwefelsaure. Folgende Ergebnisse wurden erhalten:

Nr.	Hydrolysemittel	Temperatur (°C)	Zeit	Entstehende Zuckermenge als Xylose (%)
I-1	Salzsäure vom spez. Gew. 1,21	23～25	1 Stunde	33,05
			2 Stunden	49,03
			3 "	51,41
			4 "	56,06
			5 "	58,13
			6 "	56,63
			7 "	57,06
I-2	Salzsäure vom spez. Gew. 1,20	23～25	6 Stunden	52,49
			18 "	59,28
			24 "	62,71
			48 "	65,05
I-3	5%ige Salzsäure	130～140	1 Stunde	56,93
			2 Stunden	71,20
			3 "	72,90
			4 "	72,90
			5 "	72,55

II-1	6%ige Salpetersäure	115~120	1 Stunde	62,12
			2 Stunden	64,75
			3 "	70,20
			4 "	71,91
			5 "	72,84
			6 "	72,02
II-2	3%ige Salpetersäure	115~120	0,5 Stunde	37,37
			1 "	48,20
			1,5 "	53,87
			2 Stunden	56,67
			3 "	58,83
			4 "	60,65
			5 "	68,52
			7 "	66,49
			10 "	74,64
			15 "	71,52
			20 "	68,88
III-1	10%ige Schwefelsäure	130~140	0,5 Stunde	50,97
			1 "	58,99
			2 Stunden	62,42
			3 "	66,21
			4 "	75,13
			5 "	74,08
III-2	5%ige Schwefelsäure	130~140	1 Stunde	44,73
			2 Stunden	55,49
			3 "	60,92
			4 "	58,81
			5 "	75,31
			6 "	70,38
			7 "	69,73
			11 "	71,14
			15 "	73,49
III-3	2%ige Schwefelsäure	130~140	1 Stunde	36,68
			2 Stunden	43,37
			3 "	53,52
			4 "	56,68
			5 "	62,63
			7 "	67,56
			10 "	66,14
			12 "	69,96
			15 "	68,75
			20 "	73,32
			25 "	71,10
			30 "	72,12
			35 "	71,64

Aus diesen Versuchen geht hervor, dass sich bei Verzuckerung des Xyloglukuronids nur eine maximale Ausbeute von 75% Zucker als Xylose (theoretisch 105,85%) ergab, und dass daher die Frage der quantitativen Hydrolyse noch nicht gelöst ist. Hier interessiert sie, unter welchen Umstanden das Xyloglukuronid über die Zustandsänderungen hinaus durch Hydrolyse chemisch aufgespalten wird. Bisher ist viel darüber gearbeitet worden, eine Biose, die Xyloglukuronsäure, als Zwischenprodukt, bei Verzuckerung zu erhalten. Heiderberger und Goebel [2] geben an, dass das Polysaccharid aus dem Friedländer-Bacillus Typ A, wenn es bei Gegenwart von konz. Schwefelsäure über Nacht stehen bleibt, worauf es mit Wasser einer 3%igen Schwefelsäure

versetzt und zum Sieden erhitzt, dann zu dem Disaccharid, Glukuronoglukuronsäure, umgewandelt werden kann. Nach Heiderberger und Kendall[3] entsteht bei der 20 Stunden langen Einwirkung von 2%iger Schwefelsäure auf arabischen Gummi auch ein Disaccharid, Glukuronogalaktose. Der Schleimstoff aus Ulmus fulva wird nach Anderson mit 4%iger Schwefelsäure hydrolysiert, wobei ein Disaccharid gewonnen wird, das mit Rhamnogalakturonsäure identisch ist.

Wir wollen nach dieser Ausführung im klaren sein, dass aus dem Xyloglukuronid bei der Verzuckerung auf oben angeführte 8 verschiedene Weise ein neues Disaccharid, Xyloglukuronsäure, hydrolisiert wird. Eine Rein von Isolierungsversuchen wurde weiter fortgeführt, wobei Xyloglukuronsäurebarium durch vielmalige fraktionierte Ausscheidung mit Methylalkohol abgetrennt wurde. Es löst sich nur in Wasser sehr leicht, aber nicht in anderen Lösungsmitteln; mit Methylalkohol wird ein weisses Pulver ausgefällt:

Zersp. Ca. 180°C. unter Braunfärbung, $[\alpha]_D^{20} = +54.8°$ (im Wasser).
80,730 mg Subst. (5,47% Wassergehalt) gaben 22,518 mg SO_4Ba.
$C_{22}H_{34}O_{22}Ba$ Ber. Ba 17,44%, Gef. Ba 17.36%.
0,3570 g Subst. gaben nach Lefèvre 11,73% CO_2. $C_{22}H_{34}O_{22}Ba$ Ber. CO_2 11.17%
0,2950 g Subst. gaben nach Tollens 38,41% Xylose. $C_{22}H_{34}O_{22}Ba$ Ber. Xylose 38.11%
3,993 mg Subst: 4,816 mg CO_2, 1,812 mg H_2O
3,418 mg Subst: 4,156 mg CO_2, 1,571 mg H_2O

$C_{22}H_{34}O_{22}Ba$	Ber.	C	33,52	H	4,35
	Gef.	C	32,89	H	5,08
	Gef.	C	33,25	H	5,14

Xyloglukuronsäurebarium gab bei der Oxydation mit Bromwasserstoffsäure und Brom, *d*-Zuckersäure, die dann in Form ihres sauren Kaliumsalzes identifiziert werden konnte. Eine für die Xylose charakteristische Reaktion Bertrands, welche die Bildung von bootförmigen Kristallen der Doppelverbindung Xylonsaures Cadmium-Brom-cadmium ergibt, wurde ausgeführt. Schleimsäure aus Galakturonsäure oder Galaktose, Mannosephenylhydrazon, Arabinose-*p*-Brom-phenylhydrazon wurde nicht erwiesen. Der Nachweis der Fruktose mittels der Resorcinprobe nach Seliwanoff, der alkoholische *α*-Naphtollösung nach Pinoff und der Ammoniummolybdatlösung fiel negativ aus.

10 ccm Lösung (entsprechend 176.1 mg Xyloglukuronsäure) verbrauchten an 0,1 N-Jod, 10,90 ccm 177,67 mg Aldose.

Aus dieser Reihe von Versuchen ergibt sich, dass das Disaccharid aus 1-Mol Xylose und 1-Mol Glukuronsäure aufgebaut is, wonach es also als eine Xyloglukuronsäure aufzufassen wäre.

Das Reduktionsvermögen von Xyloglukuronsäurebarium wurde wie folgt bestimmt:

Xyloglukuron-säurebarium (mg)	Xyloglukuron-säure (mg)	Kupfer (mg)	Xyloglukuron-säurebarium (mg)	Xyloglukuron-säure (mg)	Kupfer (mg)
21,0	17,39	18,85	71,9	59,54	56,16
55,0	45,54	44,73	105,6	87,45	81,23

Aus diesen Ergebnissen ist folgende Tabelle in ihrer Beziehung zu der maximalen Ausbeute der oben angeführten 8 verschiedenen Hydrolysen zusammengestellt:

Nr.	Hydrolysemittel	Zeit	Kupfer (mg)	Entstehende Zuckermenge als Xylose (%)	Zuckermenge als Xyloglukuronsäure (%)
I-1	Salzsäure vom spez. Gew. 1,21	5 Stunden	42,17	58,13	116,97
I-2	Salzsäure vom spez. Gew. 1,20	48 "	69,55	65.05	134,35
I-3	5%ige Salpetersäure	4 "	65,14	72,90	148,91
II-1	6%ige Salpetersäure	5 "	65,09	72,84	148,80
II-2	3%ige Salpetersäure	10 "	65,29	74,64	153,76
III-1	10%ige Schwefelsäure	4 "	67,04	75,13	154,47
III-2	5%ige Schwefelsäure	5 "	67,19	75,31	155,12
III-3	2%ige Schwefelsäure	20 "	64,19	73,32	150,65

Es kommt sicher vor, dass man bei Hydrolyse des Xyloglukuronids nicht nur die Bildung von Xylose und Glukuronsäure, sondern auch der Xyloglukuronsäure bewirken kann. Der Bestandteil des Xyloglukuronids ist als Xylose und Glukuronsäurederivat (1 : 1) in vollkommen gleicher Menge wie der der Xyloglukuronsäure vorhanden, dabei konnte man die Reaktion anderer Hexosen, Pentosen und Methylpentosen nicht nachweisen.

Literatur

(1) K. Nishida, H. Hashima & T. Fukamizu: Bull. Agric. Chem. Soc. Japan, **10**, 162 (1934).
(2) M. Heiderberger & W. F. Göebel: J. Biol. Chem., **74**, 613 (1927).
(3) M. Heiderberger & F. E. Kendall: J. Biol. Chem., **84**, 639 (1929); O. L. Butler & L. Cretcher: J. Amer. Chem. Soc., **51**, 1519 (1929).
(4) E. Anderson: J. Biol. Chem., **104**, 163 (1934).

Studies on "Miso" V.—On vitamin B. (pp. 272~283): By Yosito Sakurai and Iwao Iwamura. (Institute of Dietary Science, Tokyo, Japan.)

Untersuchungen über die Verwitterung der Eruptivgesteine I.—Verwitterung der Basalte (1). (pp. 283～300): Von Mitsuru HARADA. (Landwirtschaftliche Hochshule Tottori, Japan.)

On the components of Shaohsing-chiu. (pp. 301～302): Yinchang WANG. (Agricultural Chemical Laboratory, Tokyo Imperial University.)

From organic acids i. e. formic acid, acetic acid, lactic acid, succinic acid and one organic base i. e. histidine are separated from Shaohsing-Chiu and identified.

Studies on the Constituents of the Mulberry Leaves, especially on the Proteins. Part VI.—On the Difference in the Quantity of Synthesis among the Proteins in the Mulberry Leaves. (Vol. 10, pp. 767～776): By Yukitaro KISHI. (At the Katakura Research Institute of Mulberry Culture, near Hachioji Tokyo Prefecture, Japan.)

(1) I have studied the difference in the synthesis and increase among the proteins of the mulberry leaves; namely, the causes of the differences in the quality of the total proteins in accordance with the growth of the mulberry leaves.

(2) When the mulberry leaves whose synthesis of proteins was hindered and whose chlorophyll and the accompanying pigments were made to decrease, by means of the intercepted sunshine, were re-exposed to the sunshine, the protein soluble in 60% boiling alcohol containing 0.3% of sodium hydroxide is comparatively near in the synthetic quantity to the total proteins which are the same as the chief constituents of seed proteins, namely albumin, globulin, prolamin, glutelin, soluble respectively in water, 10% solution of sodium chloride, 70% alcohol, 0.2% solution of sodium hydroxide.

In this case, in accordance with the progress of exposition to the sun, or with the increase in the chlorophyll and the accompanying pigments, the former protein has become far greater in its synthetic quantity than the latter proteins.

This result is similar to the case in which the protein, soluble in the hot alkaline alcohol, is rapidly produced when the mulberry seeds which contain globulin, glutelin, etc., bud forth in the sunshine, and the chlorophyll and the accompanying pigments rapidly appear.

(3) In the mulberry leaves exposed to the sun, even when the chloro-

phyll and the accompanying pigments decreased in quantity as the case (2), the protein soluble in the hot alkaline alcohol is greater in the synthetic quantity than the proteins similar to those in seeds.

In normal circumstances, the difference of proportion of quantity among the proteins that are synthesized in the mulberry leaves in the sunshine, is greater than the difference among the proteins that formerly existed before the synthesis. The protein soluble in the hot alkaline alcohol is greater in synthetic quantity than the proteins similar to those contained in the seeds.

(4) Whether the difference in the quantity of synthesis among the proteins in the green leaves in the sunshine is only indirectly due to the sunshine or also directly due to it, is not clear. In any case, I presume, the difference in accordance with the progress of the exposition to the sun, in other words, with the lapse of days or with the development of the mulberry leaves, in the quality of the proteins in the leaves that bud forth on the stems, is due to the difference in the quantity of synthesis which is mainly caused by the sunshine as it is expressed in (2) and (3). And I also presume that this phenomenon is common to all the plants with green leaves.

Studies on the Constituents of the Mulbery Leaves, especially on the Proteins. Part VII.—On the Indirect Causes which Affect the Quantity, in the Silkworm and the Silk, of the Proteins that have been Accumulated from the Proteins in the Mulberry Leaves. (Vol. 10, pp. 1204～1210): Yukitaro Kishi. (At the Katakura Research Institute of Mulberry Culture, near Hachioji Tokyo prefecture, Japan.)

(1) I have studied the causes which indirectly affect the quantity, in the silkworm and the silk, of the proteins that have been accumulated from the proteins in the mulberry leaves. With the difference in the constituents of the mulberry leaves, I have found the different percentages of the proteins that have been accumulated from the digested proteins. And the cause of the different percentages have also been experimented in this studies.

(2) Almost all the digested proteins from the mulberry leaves ordinarily used in sericulture, have been accumulated in the silkworms that are growing up.

In accordance with the increase of the total carbohydrates to be converted by the dilute hydrochloric acid in the mulberry leaves or with the increase of the digested carbohydrates in quantity, the quantity of metabolism of the proteins in the silkworms tends to decrease, and consequently the percentage of the proteins to be accumulated in the silkworms tends to increase.

When the sugars were given to the silkworms with the mulberry leaves

the similar results were found.

In the mulberry leaves used for the fifth stage of the silkworms in August silkworm culture, as far as this experiment went, I found that the comparatively young leaves had more quantity of carbohydrates to be converted by the dilute hydrochloric acid and the percentage of the accumulated proteins in the silkworms was higher.

Studies on the Constituents of the Mulberry Leaves, especially on the Proteins. Part VIII.—On the Metabolism of the Proteins in the Silkworms when the Quantity of the Soluble Carbohydrates Contained in the Mulberry Leaves is Excessively Little. (pp. 222～231): By Yukitaro KISHI. (At the Katakura Research Institute of Mulberry Culture, near Hachioji Tokyo Prefecture, Japan.)

(1) I have studied the two different cases of the indirect causes, which are mainly due to the soluble carbohydrates to be converted with the dilute hydrochloric acid, of the difference in the quantity, in the silkworm and the silk, of the proteins that have been accumulated from the proteins in the mulberry leaves.

(2) When the soluble carbohydrates are little and the digestibility is poor, as in the youngest leaves soon after the sprouting, the quantity of the soluble carbohydrates to be digested by the silkworms is very scarce.

In such cases the scarcity of the soluble carbohydrates excessively increases the quantity of metabolism of proteins and, as a result of this, greatly decrease the percentage of the accumulated proteins in the silkworms.

In this case the indirect causes come to be the main influences which decide the quantity of proteins in the silkworms and the silk.

(3) Among the proteins contained in the youngest leaves soon after the sprouting, the total sum of globulin, glutelin, and albumin is higher, and the proportion of the protein soluble in alkaline alcohol is less, than in the more mature leaves, as I have reported in Part II*. And the digestibility of the total protein in the youngest leaves is higher or, in other words, the total proteins are better in quality than the more mature leaves.

The youngest mulberry leaves soon after the sprouting are defective because they are wanting in other constituents than the proteins, for the qnantity of the soluble carbohydrates to be digested by the silkworms is excessively little as is shown in (2). Through such indirect causes the quantity of proteins in the silkworms and the silk decreases when the worms are fed

* Y. Kishi: Bull. of Agricul. Chem. Soc. of Japan, Vol. IX, Nos. 1～3, 42 (1933).

with the youngest leaves soon after the sprouting.

(4) In accordance with the growth of the mulberry leaves the quantity of the soluble carbohydrates and their digestibility increase and then generally decrease, so the quantity of the soluble carbohydrates to be digested by the silkworms makes similar changes.

In the comparatively young mulberry leaves that are generally given in the stage of the young silkworms, the quantity and the digestibility of the soluble carbohydrates become more than the mature leaves, in accordance with the growth of the leaves. Hence it follows that the digested quantity of the carbohydrates makes an increase. Generally, however, in the silkworm culture of this stage, the younger the mulberry leaves are the more increase in the quantity of the proteins in the silkworms is made.

In the mulberry leaves generally used, though not in the youngest leaves soon after the sprouting, the direct causes, due to the proteins themselves, among all the causes from the constituents that decide the quantity of the proteins in the silkworms and the silk, would become dominant.

Studies on the Constituents of the Mulberry Leaves, especially on the Proteins. Part IX. (pp. 232～241): By Yukitaro KISHI.
(At the Katakura Research Institute of Mulberry Culture, near Hachioji Tokyo Prefecture, Japan.)

The résumé of the studies in the reports I～VIII is as follows.

[1] The Studies in the Biochemistry of Plants.

1) The mulberry leaves have a little of the proteins similar to those contained in the seeds, but much of the protein soluble in the 60% boiling alcohol containing 0.3% of sodium hydroxide.

This may be the common character of all the green leaves.

2) The distribution of the proteins in the mulberry trees is as follows:—

Proteins in reservatory organs as the seeds and the roots and the proteins in flowing juice consist in globulin, glutelin, albumin etc.

In the green leaves the existence of proteins is stated in (1). In the stems that partly possess the chlorophyll and the accompanying pigments, the proteins exist about half as much or as little as the leaves or the roots and seeds.

3) In accordance with the growth of the mulberry leaves, the difference in the quantity of each protein happens; hence the change in the quality of the total proteins happens. In other words, the proportion of the protein soluble in the alkaline alcohol against the proteins similar to those mainly found in the seeds, gets higher. This phenomenon may be understood as common character to all the green leaves of the plants. The above-mentioned

difference in the quality of the proteins may be proved by the following experiments.

A) When I estimated each protein separately, making use of its solubility, and compared with one another, I found the difference in the quality in the total proteins in accordance with the growth of the mulberry leaves.

B) By the degrees in the growth of the mulberry leaves, I found different digestibility in the total proteins. The younger the leaves, the higher the digestibility. And the youngest leaves soon after the sprouting showed the highest digestibility. The difference of digestibility may be proved as follows.

(1) By the degrees in the growth of the mulberry leaves, the digestibility of the total proteins showed difference both in the experiment with the silkworms and in the artificial digestion with the gastric juice of the silkworms. And the younger leaves showed the better digestibility.

(2) In the experiment of the artificial digestion with the gastric juice of the silkworms, the same result was obtained either with the air-dried powders of the mulberry leaves or with the well-crushed materials of the fresh leaves or with the juice, containing the whole proteins, taken from the fresh mulberry leaves by means of crushing and then pressing them well. In other words, the younger leaves showed the better digestibility of the total proteins.

(3) When the buffer solution with the same pH was added to the mulberry leaves with different degrees of growth, the different values of pH were found. Even when the latter pH was made of the same value, the conclusion of the experiments in digestibility was the same as the fore-mentioned cases, (1) and (2), in accordance with the growth of the leaves.

(4) In the experiment of artificial digestion of each protein prepared from the leaves, the seeds, and the roots of the mulberry trees, with the gastric juice of the silkworms, the protein soluble in the alkaline alcohol is worse in digestibility than the proteins similar to those contained chiefly in seeds. In accordance with the growth of the leaves, the proportion of the protein soluble in the alkaline alcohol, in the total proteins, against the proteins similar to those mainly contained in the seeds, becomes higher, as it is mentioned in 3). Hence it follows that the digestibility of the total proteins of the mulberry leaves, differs in accordance with the growth of the leaves.

C) The difference in the quantity of each protein synthesized in the mulberry leaves in the presence of the sunshine and the chlorophyll and the accompanying pigments, and the synthetic quantity of the protein soluble in the alkaline alcohol is greater than the proteins similar to those mainly contained in the seeds. And the difference between the synthetic quantity of the protein soluble in the alkaline alcohol and the proteins similar to

those mainly contained in the seeds becomes greater in accordance with the increase in the chlorophyll and the accompanying pigments and the sunshine. As the quantity of the chlorophyll and the accompanying pigments is generally in direct proportion to the quantity of the sunshine, the difference in the quantity of synthesis and increase of each protein in the mulberry leaves depends upon the quantity of the sunshine.

This result is similar to the fact that the quantity of the proteins remarkably changes after the seeds of the mulberry trees bud forth in the sunshine and come to contain the chlorophyll and the accompanying pigments. Hence it follows that the proteins in the mulberry leaves differ in quality in accordance with the increase of of the sunshine or the lapse of time or the growth of the leaves after the leaves bud forth on the stems.

4) The percentage of the proteins contained in the fresh leaves gradually becomes lower, but, to be more precise, there are other cases owing to the different conditions of the mulberry leaf-growing such as the seasons of the cutting of the trees, the periods of the use of the leaves, the kinds of the trees or the characters of the soil. Therefore the quantity of the proteins in the fresh leaves may first increase and then decrease in accordance with the growth of the leaves.

[2] Studies in the Chemistry of Sericulture or in the Constituents of Mulberry Leaves which are the Main Causes for the Difference in the Quantity of the Proteins in the Silkworms and the Silk.

The causes, to be found in the constituents of the mulberry leaves, for the difference in the quantity of the proteins in the silkworms and the silk when the same quantity of the mulberry leaves is given the silkworms, may be various, but the main causes are as follows:—

1) The direct causes to be found in the proteins contained in the mulberry leaves:—

(1) The difference in the digestibility of the proteins in the mulberry leaves is caused by the difference in the quality of the total proteins in the leaves. In accordance with the growth of the mulberry leaves, the younger the leaves, the greater the digestibility of the total proteins.

(2) The difference in the total proteins contained in the fresh mulberry leaves.

The difference in the percentage of the total proteins in the fresh mulberry leaves means the difference in the quantity of the total proteins given the silkworms when the quantity of the leaves given is the same. The quantity of the proteins given the silkworms may generally decrease in accordance with the growth on the mulberry leaves, but, to be more precise, we found other cases on account of the different conditions of the mulberry-leaf growing such as the seasons of the cutting of the trees, the periods of the use of the leaves, the kinds of the trees or the characters of the soil.

(3) The difference in the percentage of the quantity of the proteins, in the mulberry leaves, to be ingested by the silkworms.

Generally speaking, and especially in the mulberry leaves used in the fifth stage of the silkworms, there is a tendency that the younger leaves are higher in the percentage of the quantity of the proteins to be ingested by the silkworms.

2) The indirect causes.

This means the causes that have recourse to constituents other than the proteins contained in the mulberry leaves. Although there may be various indirect causes, the quantity of the soluble carbohydrates to be converted in the dilute hydrochloric acid is the main one. This cause has two different cases.

(1) When it is the main cause for the difference in the quantity of the proteins contained in the silkworms and the silk:—

When the quantity of the soluble carbohydrates is very little in the youngest leaves soon after the sprouting, the scarcity is substituted by the proteins, and the metabolism of proteins in the silkworms remarkably increases. As a result of this, the percentage of the remaining proteins in the silkworms becomes excessively lower and such defects become the main causes for the decrease in the proteins contained in the silkworms and the silk, though the proteins in the youngest leaves are better in quality than the more mature leaves.

(2) When the mulberry leaves are ordinarily used in sericulture:—

In this case the more the quantity of the soluble carbohydrates in the leaves, the higher the percentage of the remaining proteins in the silkworms, but it does not come to be the main cause for the difference in the quantity of the proteins contained in the silkworms and the silk, for the main cause is in the direct causes to be found the proteins contained in the mulberry leaves.

Judging from the fore-mentioned points, it may be remarked that the difference in the quantity of the proteins contained in the silkworms and the silk depends upon the algebraic sum total of the above-mentioned various causes in the constituents of the mulberry leaves. And the fact that in the ordinarily used in sericulture, the younger leaves increase more the quantity of the proteins in the silkworms and the silk, is due to the greater algebraic sum total, or in other words, mainly to the better quality of the proteins themselves.

Studies on the Constituents of the Mulberry Leaves, especially on the Proteins. Part X.—Studies on the Quantity of Sericin and

Fibroin in the Silk by the Difference in the Constituents of the Mulberry Leaves in accordance with the Growth of the Leaves. (pp. 303~309): By Yukitaro KISHI. (At the Katakura Research Institute of Mulberry Culture, near Hachioji Tokyo Prefecture, Japan.)

(1) Owing to the difference in the constituents of the mulberry leaves in accordance with the growth, the difference occurs in the proportions of sericin and fibroin in silk. In the mulberry leaves ordinarily used in sericulture, the younger leaves have less sericin, but more fibroin than the mature leaves.

(2) The proportion of sericin and fibroin differs mainly by the difference in the constituents of the leaves given in the fifth stage of silkworms. There may be various causes for this, as far as the constituents of the mulberry leaves are concerned, but the main cause may be in the difference of quality of the total proteins contained in the mulberry leaves.

(2) When the soluble carbohydrates to be converted by the dilute hydrochloric acid is excessively little, as in the youngest leaves soon after the sprouting, the quantity of sericin increases. When sugars are given together with the youngest leaves, the quantity of silk increases, sericin decreases and fibroin increases. Therefore the scarcity of the soluble carbohydrates may be the main cause for the increase of sericin and the decrease of fibroin in silkworms fed with the youngest leaves.

(4) In the case (3) where the scarcity of the soluble carbohydrates are substituted with the proteins in metabolism when the soluble carbohydrates are very little as in the youngest leaves, we presume that the proteins and amino acids that are convenient for the synthesis of fibroin are mostly made use of. In the cases (2) and (3) we presume that the younger the leaves, the more the proteins convenient for the synthesis of fibroin in the silkworms.

Über den Schleim von Scaphium affine pierre. (pp. 310~315): Von Hikonojo NAKAHARA. (Aus den Agrik. chemischen institute der kaiserlichen Universität zu Tokio.)

Die Frucht von Scaphium affine pierre (Sterculiaceae) gedeiht in Siam und auf der Maleischen Halbinsel und wird in Japan als Zuspeise benuzt.

Es galt für mich festzustellen, aus welchen Bestandteilen das Fruchtfleisch besteht, das beim Hineinlegen der Frucht ins Wasser, spontan und leicht aufquellt und als gallertartige Substanz ihrer dünnen Schalen entfliesst. Nach 1.5 stündigem Erhitzen im Autoklave auf 130°C löst sich der gallertartige Schleim im Wasser vollständig auf. In dieser Lösung entsteht durch Zusatz von 95 proz. Alkohol ein flockiger Niederschlag, der für ein dem Ehrlichschen Ca–Mg–Salz der Pektinsäure entsprechendes Salz gehalten wird.

Der Niederschlag wurde mit Salzsäure und Alkohol behandelt, und die erhaltene aschegeringe, saure Substanz (Pektinsäure) bei der Hydrolyse mit Schwefelsäure lieferte Galakturonsäure (37,28%), Galaktose (26,44%), Arabinose (26,23%) und Essigsäure (8,67%), jedoch bei der Hydrolyse derselben mit 2 proz. Salzsäure weder Tetragalakturonsäure a noch b.

The Influence of Intermittent Supply of Protein on the Growth of Albino-rats (I). (pp. 316~320): By Rinjiro SASAKI and Norihide ANDŌ. (Agr. Chemical Laboratory, Tokyo Imperial University.)

Monoamino Acids of Soj-bean Protein. (pp. 321~331): Shûiku SASAKI. (From the Biochem. Laboratory, Depart. of Agr. Kyûshû Imp. Univ.)

The whole protein was prepared from soj-beans.

The protein was hydrolyzed by boiling with sulfuric acid, and the resulting monoamino acids determined by more recent methods.

TABLE I.—*Result of Fractionation of Monoamino Acids of Soj-bean Protein.*

Amino acid	Form in which separated	% of wt. of protein	% of total N. (16.6% N in protein)
Glycine	Picrate	0.23	0.26
Alanine	Copper salt	4.12	3.90
Valine		2.56	1.84
Leucine		10.02	6.45
Isoleucine	Copper salt	2.38	1.53
Proline	Picrate	3.94	2.86
Phenylalanine	Hydrochloride and Benzoic acid	5.21	2.66
Tyrosine		3.82	1.78
Aspartic acid	Copper salt	15.09	3.23
Glutamic acid	Hydrochloride	16.50	9.44
Oxyglutamic acid		13.20	6.87

On Systematic Study of Alcohol and Carbohydrate Oxidizing Bacteria isolated from Fruits, and a New Classification of the Oxidizing Bacteria. (Continued) (pp. 331~340): Toshinobu ASAI. (Agr. Chem. Laboratory, Imp. University of Tokyo.)

Further Purification of Biosterin and its Crystalline Derivative. (pp. 341~345): By Sadayuki HAMANO. (Institute of Phy. and Chem. Research.)

On a Crystalline Derivative of Vitamin A. (pp. 346~348): By Sadayuki HAMANO. (Institute of Physical and Chemical Research.)